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(10) CANADIAN PATENT

(54) APPARATUS FOR THE CONCENTRATION OF MACROMOLECULES AND SUBCELLULAR PARTICLES FROM DILUTE SOLUTIONS

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No. OF CLAIMS 7

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This invention relates to centrifuge systems adapted to process large volumes of liquid flowing continuously from a suitable source such as a reservoir.

Centrifuge systems of this type have been known for some time. An example of such system is disclosed in U.S. patent No. 2,834,541 issued May 13, 1958. In such a system, large volumes of liquids which produce comparatively small masses of precipitates are centrifuged essentially continuously. Such system comprises a plurality of apparatus arranged around a rotor, a liquid containing reservoir positioned above the rotor, a plurality of inlet tubes for communicating between the reservoir and each of the apparatus, and a like plurality of outlet tubes through which the excess centrifuged solution may flow.

The invention relates to an improvement over the above mentioned apparatus used in the centrifuge system disclosed which permits the rapid concentration and fractionation based on molecular size under mild conditions of protein and other macromolecules from large volumes of dilute solutions. The improved apparatus may also be used for the concentration of subcellular particles such as ribosomes and viruses under similar conditions.

The apparatus, in accordance with the invention, comprises an outer tube and an inner tube positioned within each outer tube in such a way as to leave a space between the outside of the inner tube and the inside of the outer tube. Each inner tube comprises at least two superposed sections having filter means dividing the sections. The bottom section of the inner tube has holes therein in communication with the space between the inner and outer tubes. A cap closing both the inner and outer tubes has two apertures therein, one in communication with the inner tube for permitting the dilute solution to enter the inner tube, and the other in communication with the space between the inner and outer tubes for permitting the excess centrifuged solution to flow out of the apparatus.

The invention will now be disclosed with reference to the accompanying drawings which illustrate, by way of example, an embodiment of the invention and in which:

Figure 1 illustrates an exploded view of the apparatus in accordance with the invention;

Figure 2 illustrates the apparatus of Figure 1 with the various parts thereof assembled together;

Figure 3 illustrates a section view taken along line 3-3 of Figure 2;

Figure 4 illustrates a section taken along line 4-4 of Figure 2; and

Figure 5 illustrates the apparatus in accordance with the invention positioned in a rotatable rotor.

Referring to Figures 1 and 2, there is shown an apparatus comprising an outer tube made of stainless steel and an inner tube, designated generally by reference numeral 12, positioned within the outer tube in such a way as to leave a space therebetween. Inner tube 12 comprises a series of sections 14, 16 and 18 also made of stainless steel and connected together by threaded joints or couplings.

The upper section 14 of inner tube 12 is generally tubular and includes an annular ring 20 adjacent to the outside lower end thereof. The lower end of upper section 14 is partially closed and provided with a large axial opening 21. An internally threaded coupling 22 having a turned-in portion 24 slides over the outside wall of tubular section 14 and engages the edge of annular ring 20. Coupling 22 has a plurality of longitudinal projections 23 on the outer diameter thereof which fit snugly inside outer tube 10 while permitting circulation of liquid between the inner and outer tubes through the gap between projections 23.

The following section 16 of inner tube 12 is also

generally tubular and is threaded around the upper end thereof for receiving coupling 22. The upper end of tube 16 is partially closed and is provided with a large axial opening 26 having concentric flat shoulders 28 and 30. A stainless steel plate 32 perforated with fine holes rests on shoulder 28 and supports a disc of porous material 34 which acts as an immediate support for a semipermeable membrane 36 which rests on shoulder 30.

The lower end of section 14 also includes an integral annular portion 38 around which an O-ring 40 may be positioned for providing a liquid-tight seal between sections 14 and 16. A second disc of porous material 42 is inserted within annular portion 38 and overlies the top of the semipermeable membrane 36. In addition, the upper end of section 16 includes a tongue 44 adapted to engage a slot 45 in the annular ring 20 of section 14.

In assembling the two sections 14 and 16 together, as it may be seen perhaps more clearly in Figure 1, the support plate 32 and disc 34 are positioned on shoulder 28 of section 16 and semipermeable membrane 36 is positioned on shoulder 30. The O-ring 40 and the disc 42 are assembled to the lower end of section 14 and the two sections 14 and 16 are subsequently assembled together, with tongue 44 engaging slot 45, by means of threaded coupling 22. The O-ring 40 is then compressed between the lower end of section 14 and the semipermeable membrane 36 seated on shoulder 30 to form a liquid-tight seal between the two sections.

Section 16 is also provided with projections 46 to help in tightening such section on the upper section 14.

The lower end of section 16 is also threaded for receiving the bottom section 18 which is internally threaded at the upper end thereof. The bottom section 18 has a circular shaped shoulder 47 adjacent to the upper end thereof for receiving the edge of a stainless steel saucer-shaped plate 48 the bottom of which is perforated with fine holes. The bottom of saucer-shaped

plate 48 holds a disc of porous material 50 which supports a semipermeable membrane 52 positioned over the edge of plate 48.

The bottom end of section 16 is partially closed and provided with an axial large opening 54 having a shoulder 56 for receiving a disc of porous material 58 adapted to contact semipermeable membrane 52. The lower end of section 16 also has two concentric annular portions 62 and 63 between which is inserted an O-ring 64 for providing a liquid-tight seal between sections 16 and 18.

Lower section 18 has a plurality of holes 66 therein in communication with the space between outer tube 10 and inner tube 12. In addition, a number of longitudinal projections 67 are provided on the outside of section 18 so as to permit such section to fit snugly inside outer tube 10 while permitting circulation of liquid between the inner and outer tubes through the gaps between projections 67.

The saucer-shaped plate 48 is provided with a tongue 68 adapted to engage a slot 70 in the lower end of section 16 so as to prevent rotation of plate 48 with respect to section 18 during the assembly of the apparatus.

In assembling the lower section 18 to the preceding section 16, plate 48 is positioned on shoulder 47 in section 18, disc 50 is inserted within the bottom section of plate 48, and the semipermeable membrane 52 is positioned over the edge of plate 48. O-ring 64 and disc 58 are assembled to the lower end of section 16 and slot 70 of section 16 is then aligned with tongue 68 of plate 48 in section 18. Bottom section 18 is subsequently threaded on section 16 and O-ring 64 is compressed between the lower end of section 16 and membrane 52 so as to form a liquid-tight seal between sections 16 and 18.

A cap 80 closes the upper end of tubes 10 and 12 and includes two O-rings 82 and 84 contacting the inside of outer

and inner tubes 10 and 12 respectively for hermetically closing such tubes. Cap 80 is made in two sections held together by a bolt 85 having a head the diameter of which corresponds to the inside diameter of section 14 of inner tube 12. The two O rings 82 and 84 are inserted in suitable shoulders in the lower portions of the two sections of the cap. After cap 80 has been inserted in the tubes, bolt 85 is tightened to compress the O rings and insure that the cap 80 will not come out during operation of the apparatus under the pressure built up in the tubes. In addition, two apertures are provided in cap 80 for receiving inlet tube 86 and outlet tube 88. As illustrated more clearly in Figure 4, cap 80 is also provided with a slot 82 for communicating outlet tube 88 with the space between outer tube 10 and inner tube 12.

In operation, the apparatus disclosed in Figures 1 to 4 is positioned in a centrifuge system such as disclosed in the above mentioned patent No. 2,664,541 the rotor of which is illustrated schematically in Figure 5 of the drawings by reference numeral 90. A small finger member 92 attached to cap 80 prevents the rotation of the apparatus with respect to rotor 90. A dilute solution contained in a reservoir located above rotor 90 flows inside inlet tube 86 protruding through cover 94 of the rotor 90. When the rotor is set into operation, the liquid is forced down through the semipermeable membranes 36 and 52 under the influence of gravitational forces and the bottom section 18 is filled with the centrifuged liquid while the macromolecules and subcellular particles are retained by the membranes 36 and 52. Such membranes are usually of decreasing pore size and the larger particles are retained by the upper membrane 36 while the finer ones are retained by the lower membrane 52. Upon filling, the excess liquid will pass through the holes 66 in lower section 16 and fill the space between the inner and outer tubes. As this space

fills further ... liquid ... out of the system via the outlet tube ... because of the pressure head created by the reservoir holding the liquid above rotor ... New liquid to be filtered enters from the reservoir via the inlet tube 66 and the circuit here ... continuous.

Although the inner tube of the apparatus disclosed contains three sections, it is to be understood that an inner tube with only two sections with a single membrane in between is also envisaged. In addition, the system may be adapted to fractionation and concentration by dividing the inner tube into more than three ... sections and by interposing membranes of decreasing pore size, if necessary, between each section.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. An apparatus for the centrifugal concentration and partial fractionation of macromolecules and subcellular particles from a dilute solution comprising:

a) an outer tube;

b) an inner tube positioned within said outer tube in such a way as to leave a space between the outside of the inner tube and the inside of the outer tube, said inner tube consisting of at least two superposed sections having filter means dividing the sections, and the lower section of the inner tube having holes therein in communication with said space in between the inner and outer tubes; and

c) a cap for closing both said inner and outer tubes, said cap having thereon an inlet in communication with said inner tube and an outlet in communication with said space between the inner and outer tubes.

2. An apparatus as defined in claim 1, further comprising threaded means for connecting adjacent sections together and an O-ring positioned between adjacent sections for providing a liquid-tight seal between said adjacent sections.

3. Apparatus as defined in claim 2, wherein said threaded means have a number of projections distributed on the outside diameter thereof which fit snugly within the inside of the outer tube but permits passage of liquid between the projections.

4. Apparatus as defined in claim 1, wherein said lower section is threaded on the preceding section and further comprises an O-ring for providing a liquid-tight seal between the lower section and said preceding section.

5. Apparatus as defined in claim 1, wherein said filter means comprises a semipermeable membrane interposed between two discs of porous material.

6. Apparatus as defined in claim 5, further comprising a support perforated with fine holes mounted on the upper end of each following section for supporting said filter means.

7. Apparatus as defined in claim 1, further comprising O-rings around said cap for hermetically closing said inner and outer tubes.

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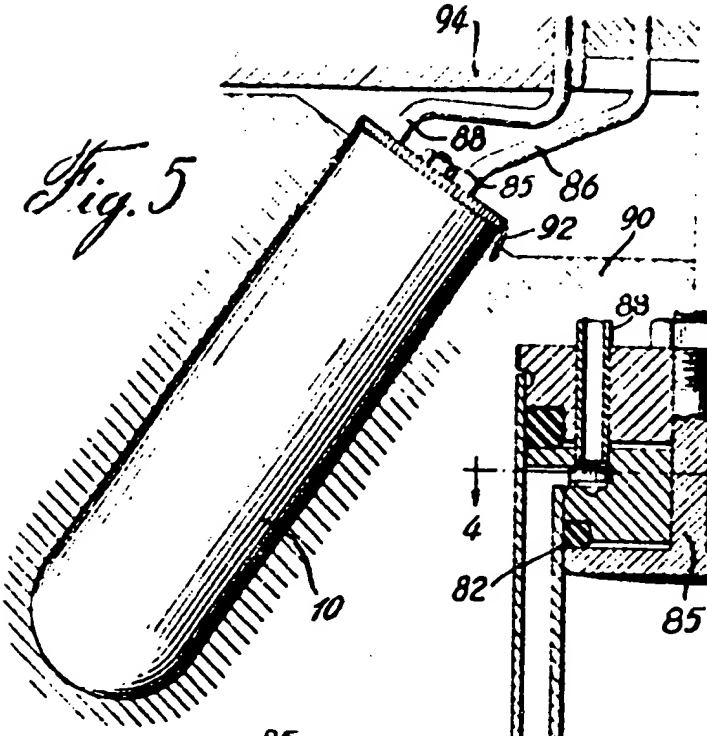

Fig. 5

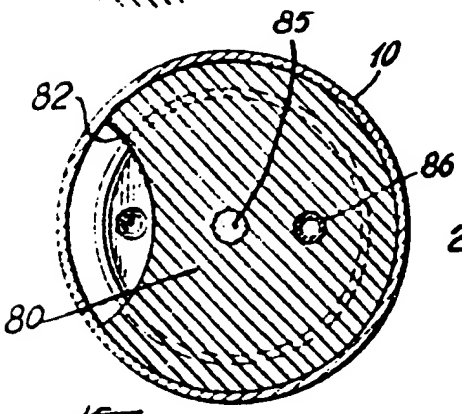

Fig. 4

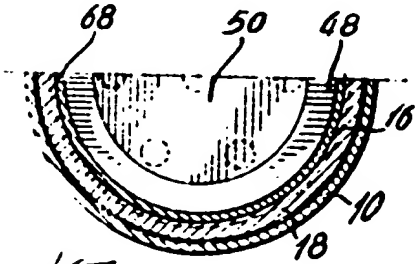

Fig. 3

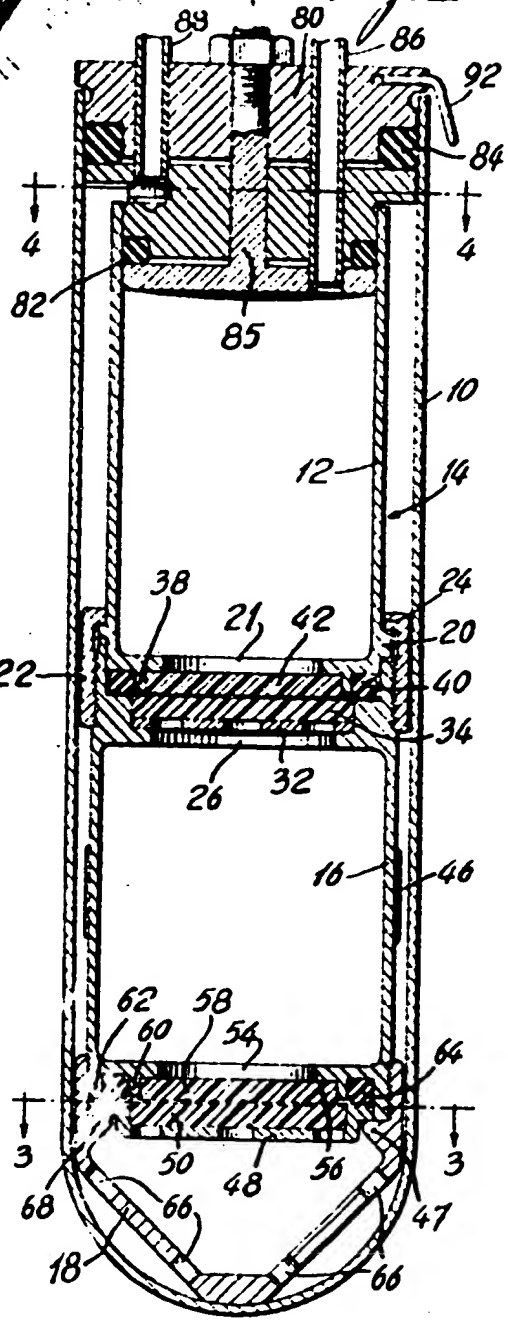

Fig. 2

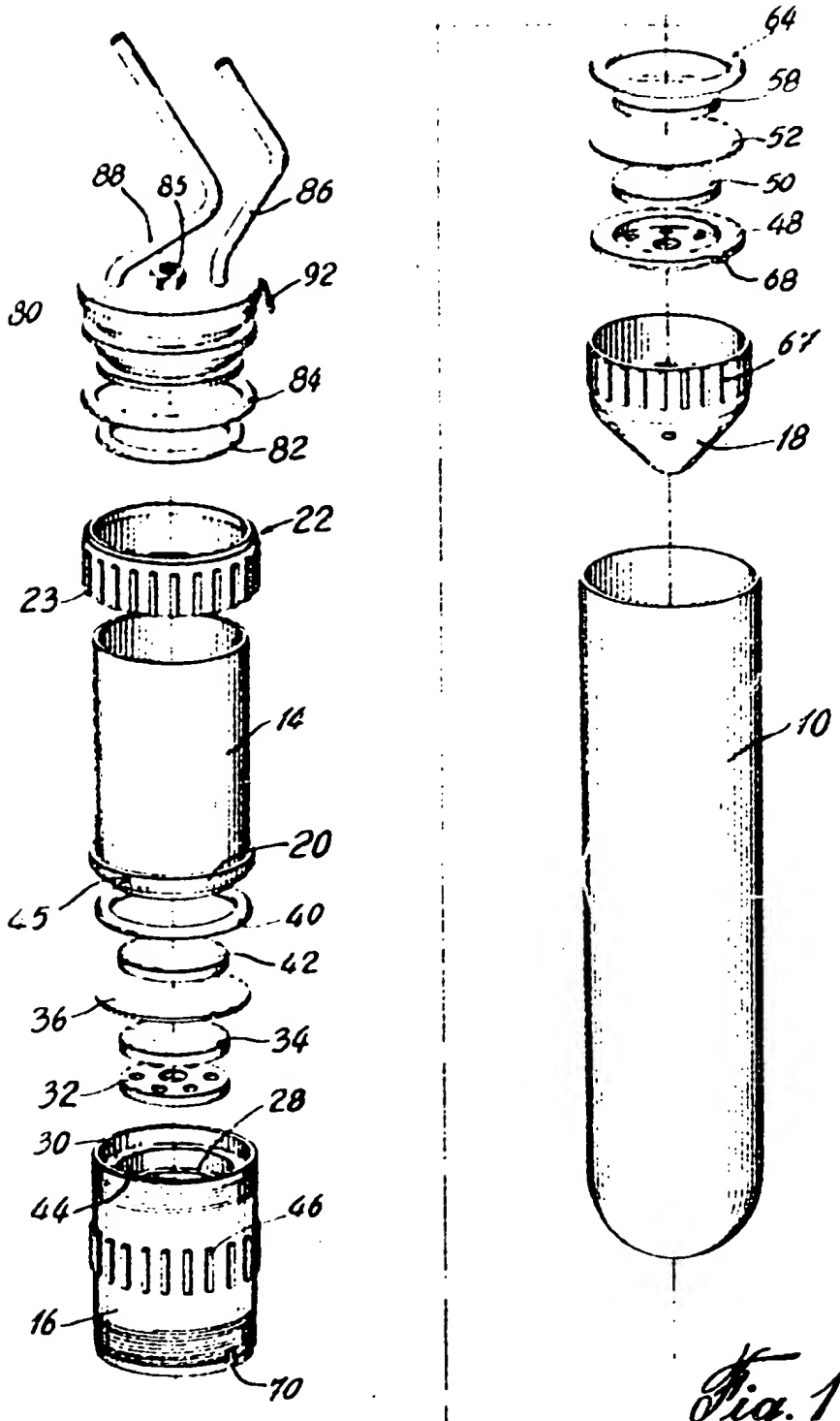
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Fig. 1